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Award Number: W81XWH-06-1-0640

TITLE: Veterinary Research Manpower Development for Defense

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REPORT DATE: September 2008

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-09-2008	Annual Summary	14 Aug 2007 – 13 Aug 2008

4. TITLE AND SUBTITLE

Veterinary Research Manpower Development for Defense

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0640

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Tufts College
Boston, MA 02111

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT The long-term goal of this training program is to increase veterinary research manpower by providing research training to veterinary students. The proposed training program included joint degree programs (DVM/MPH, DVM/MS-CBS and DVM/MS-LAM) and the summer research program. The 13 trainees recruited in the joint degree program are continuing their training and the 12 trainees, recruited in the summer research program, have completed their research projects. One trainee joined the US Army. Abstracts, but no peer-reviewed publications, have resulted from the research conducted by the trainees. No major problem was encountered with recruiting or maintaining trainee interests in the training program during the reporting period and hence we plan to continue the program as originally proposed.

15. SUBJECT TERMS
Veterinary Research Manpower, Joint-degree program, Summer Research Program

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	16	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION:

The long-term goal of this training program is to increase veterinary research manpower by providing research training to veterinary students. The program is expected to develop a pipeline of research ready veterinarians to solve biodefense and public health problems facing our country, in general, and our armed forces in particular. The following training programs offered by Cummings school of veterinary medicine were proposed for this purpose.

1. Summer Research Program
2. Four-year joint DVM/Master's degree in Public Health degree (DVM/MPH)program
3. Four-year joint DVM/Master of Science degree in Laboratory Animal Medicine (DVM/MS-LAM)
4. Five-year joint DVM/MS in Comparative Biomedical Science (DVM/MS-CBS)

During the summer research program, veterinary students are trained to conduct mentored research under the guidance of a faculty. Veterinary students are also recruited in joint degree programs that allow more specialized training in public health and laboratory animal medicine and more in-depth research training in comparative biomedical sciences.

Body:

This is a training grant with training opportunities in multiple programs, and this progress report includes accomplishment from August, 2007 to August 2008 (second year) of this multi-year program.

Program Implementation: As proposed in the application, veterinary students were informed of the training program soon after receiving the award notice. A website as well as a brochure was created to inform veterinary students and faculty (http://www.tufts.edu/vet/researchtraining/defense.html). The website included the program description, responsibilities of trainees and mentors, list of program faculty and individual program directors and an application package. In addition, students and faculty were informed of the program via email with reference to the website and a seminar was held to explain the program to students. Prospective trainees were asked to contact program faculty for summer research projects and program directors for the joint-degree programs.

Recruitment: The goal of the program during the reporting period was to recruit 12 trainees in the Summer Research Program, 4 trainees in DVM/MPH, 6 trainees in DVM/MS-LAM and 3 trainee in DVM-MS-CBS programs. Twenty students applied for the 12 trainee positions for the Summer Research Program, 6 students applied for the DVM/MPH, 7 students applied for the DVM/MSLAM program and 3 students applied for the DVM/MS-CBS program. Because of limited positions in each of the programs, 12 trainees out of 20 applicants for the Summer Research Program, 4 out of 6 applicants for the DVM/MPH program and 6 out of 7 applicants for the DVM/MS-LAM program were selected based on the scientific merit of the proposal, academic standing in the veterinary school, relevance to biodefense and stated interest in an army career. Of the three applicants, two were recruited to start the DVM/MS-CBS program in 2007 and one in 2008. One of the recruit (Elliot Gerber) is a recipient of US Army Health Professional Scholarship Program. All recruited trainees in various combined degree programs are listed in table 1, and in the summer research program are listed in table 2.

Progress to date: All trainees in the DVM/MS-LAM and DVM/MPH are still in the program, are in good academic standings and are expected to complete their training in years listed in table 1. Of the three trainees currently in the DVM/MS-CBS program, one has completed the research and submitted a thesis (details below), one is in the process of preparing the thesis and one has started the program this year. All 12 trainees in the summer research program have completed their research projects (Table 2) and are in the process of preparing research reports due by the middle of September, 2007. These trainees will present their research work at the annual veterinary student research day on October 8, 2008. They will also compete for three awards based on the written report and the oral presentation, and will receive the award at the end of the annual veterinary student research day.

Table 1: Trainees recruited in 2007 and 2008 in various combined degree programs

A. DVM/MS-LAM Program

Name of Trainee	Training Program	Training Completion Year	DVM Graduation Year	Comments
Kristina Asselin	DVM/MS-LAM	2010	2010	
Jessica Connolly	DVM/MS-LAM	2009	2009	Joined Army in 2007
Amanda Graveline	DVM/MS-LAM	2011	2011	
Amory Koch	DVM/MS-LAM	2011	2011	
Wenjun Li	DVM/MS-LAM	2009	2009	
Lindsey Miller	DVM/MS-LAM	2010	2010	
Mayrav Moreshet	DVM/MS-LAM	2009	2009	
Melissa Timm	DVM/MS-LAM	2010	2010	

B. DVM/MPH Program

Name of Trainee	Training Program	Training Completion Year	DVM Graduation Year	Comments
Karen Alroy	DVM/MPH	2011	2011	
Katherine Haman	DVM/MPH	2012	2012	
Tammy Han	DVM/MPH	2009	2009	
Miranda Hillyard	DVM/MPH	2010	2010	
Jennifer McRobbie	DVM-MPH	2010	2010	
Misha Robyn	DVM/MPH	2009	2009	
Marieke Rosenbaum	DVM-MPH	2011	2011	

C. DVM/MS-CBS Program

Name of Trainee	Training Program	Training Completion Year	DVM Graduation Year	Comments
Elliott Garber	DVM/MS-CBS	2008	2010	Thesis in progress
Marieke Rosenbaum	DVM/MS-CBS	2009	2011	Thesis started
Karyn VonIderstein	DVM/MS-CBS	2008	2010	Thesis completed

Each trainee in the Summer Research Program is required to complete a survey to provide feedback on their research experience and future plan. The survey data for the reporting period will be available after the due date of this progress report and will be included in future progress reports. The survey data from seven trainees last year revealed the following: 100% had a stimulating research experience and plan to obtain more research experience while in veterinary school, 42% considered entering a combined degree program and 71% were interested in getting involved in research after completing the DVM program.

All trainees were required to and attended a special information session on career opportunities in the US Armed forces for veterinarians. This session was organized by the local US Army recruiting officers and was held on October 29, 2007. Please note that one of the 2007 trainees (Ms. Rebecca Steers) has joined the Army during the academic year.

Table 2: Trainees recruited in 2008 Summer Research Program

Name of Trainee	Mentor	Project Title
Karen Alroy V'11	Dr. Ellis	Herring Gulls, *Larus argentatus*, as vectors of pathogenic *Escherichia coli* in coastal environments
Sarah Carter V'10	Dr. Kaufman	Determining Geographic Risk Factors for Disease Transmission between Livestock and Elephants in Chitwan, Nepal
Philip Gerwin V'11	Dr. Mann	The Role of Progesterone and Its Metabolites on the Onset of Maternal Behavior during Pregnancy in Rats

Sara Heslop V'11	Dr. Maranda	Prevalence of Gastro-intestinal Parasites in White-faced Capuchin Monkeys, *Cebus capuchinus,* in Heavily Touristed National Parks in Costa Rica Compared to Isolated National Parks.
Miranda Hillyard V'10	Dr. Alders	Aflatoxicosis in Indonesian domestic ducks: implications for H5N1 highly pathogenic avian influenza (HPAI) vaccination campaigns.
Lauren O'Connell V'11	Dr. Bridges	Estrogen, Maternal Behavior and Stress
Kathleen Riley V'10	Dr. Kaufman	Diagnosis and Treatment of Mange in the Street Dog Population of Kathmandu, Nepal
Annie Shea V'11	Dr. Shaw	The Use of Directed Education to Improve the Rate of Hand Washing in a Veterinary Hospital
Angela Snell V'11	Dr. Alders	A Participatory Approach to Understand Poultry Farmer Perceptions of Avian Influenza (H5N1).
Christie Taylor V'11	Dr. Daszak	Investigating the Emergence of SARS and SARS-like Coronoviruses.
Stephen Wilson V'11	Dr. Epstein	Infectious Risks of Brushmeat Hunting on Malaysia
Tierra Wilson V'10	Dr. Mukherjee	Development of a Saliva Collection Technique for Improved Health-Monitoring of Free-Living Mountain Gorillas.

Key Research Accomplishments

The goal of this program is to provide research training to veterinary students by allowing them to participate either in a research project over the summer months (Summer Research Program from June to August) or a year-long hypothesis driven research (DVM/MS-CBS). The short term summer research training usually does not result in the publication of a manuscript, as the trainees are still learning the research methods and the project is usually not completed during the summer months. Thus, the focus is to get the trainees interested in research including how to evaluate and prepare scientific publications. Since the final report of the 2008 summer research accomplishments is not due until the middle of September, 2008, i.e., after the due date of this progress report, key research accomplishment will be reported in the next annual progress report. Thus, the research accomplishment of 2007 summer research is included in this report (see appendix 1). The 2008 summer research trainee and the project titles are summarized in table 2, and the summary of each project is listed in appendix 2. Key training areas of the Summer Research trainees can be summarized as follows:

1. Construction of global positioning system map to describe the distribution of bovine rabbies.
2. Immunohistochemical testing for rabbies
3. PCR technique to detect bat and viral DNA
4. Seroprevalence of antibodies to Australian bat lyssavirus
5. Rose Bengal agglutination test to detect seroprevalence of Brucellosis melitensis
6. Participatory disease surveillance method for the detection of highly pathogenic avian influenza
7. Development of multiplex PCR technique for differentiating among three species of mycobacterium.
8. Comparison of tuberculin test and nested PCR to detect bovine tuberculosis

In addition to summer research trainees, two trainees participated in the year-long hypothesis driven research in 2008. One of those two trainees has completed the project and the other trainee is in the process of finalizing the thesis. A summary of the completed project is shown below:

Stem cell antigen-1 (Sca-1), a murine-specific stem cell marker, plays a role in the regulation of proliferation and differentiation of progenitor cells during tissue repair. Sca-1 has been used as a marker to isolate pulmonary stem cells, including the bronchioalveolar stem cell (BASC), which displays progenitor cell properties in vitro and increases in abundance during lung regeneration in vivo. To determine the role of Sca-1 during lung regeneration, we performed pneumonectomy (PNY) in 1) C57Bl/6 mice, which express high levels of Sca-1 in bone marrow, 2) BALB/c mice, which have markedly lower Sca-1 expression in bone marrow, and 3) Sca-1$^{+/EGFP}$ reporter mice, which are heterozygous for Sca-1 and express enhanced green fluorescent

protein (EGFP) targeted to the Sca-1 locus. Lung function, BASC abundance, and Sca-1 expression were analyzed in each strain following PNY. Our studies showed that while C57Bl/6, BALB/c, and Sca-1$^{+/EGFP}$ mice regrowed their lungs following PNY, there were differences in this process between strains. Sca-1 (or GFP) expression increased following PNY and both BALB/c and Sca-1$^{+/EGFP}$ strains show a decrease in BASC abundance relative to C57Bl/6. These results provide justification for further studies to determine the importance of Sca-1 (using a null model) as well as BASC in lung regeneration.

The results of the above mentioned studies have been presented in abstract forms in national meeting as listed below:

VonIderstein K, Mazan MF, Hoffman AM. Searching for SCA-1: stem cell antigen-I expression during lung regeneration in C57B16 mice. Veterinary Comparative Respiratory Society Meeting, October, 2007.

VonIderstein K, Mazan MF, Gruntman, A, Sever, L, Costa, L., Hoffman AM. Enhanced green fluorescence protein targeted to stem cell antigen-1: Linking the BASC to the BASC. Merck-Merial-NIH National Veterinary Scholars Symposium, August, 2008.

Reportable Outcomes:

1. Four (4) trainees were recruited in the DVM/MPH joint degree program
2. Six (6) trainees were recruited in the DVM/MS-LAM joint degree program
3. Three (3) trainees were recruited in the DVM/MS-CBS joint degree program
3. Twelve (12) trainees were recruited in the summer research program
4. One (1) trainee (Rebecca Steers, 2007 summer research trainee) joined the US Army in 2008.
5. One trainee completed a thesis as a partial requirement for the degree of DVM/MS-CBS.

Conclusion

The second year of this multi-year program is considered to be successful, as evidenced by the increasing number of trainees recruited in the program. All trainees in the combined degree program are making satisfactory progress. One trainee completed a thesis and another is at the final stages of thesis preparation as a partial requirement for the degree of DVM/MS-CBS. Abstracts, but no peer-reviewed publications, have resulted from the research conducted by the trainees. No major problem was encountered with recruiting or maintaining trainee interests in the training program during the reporting period and hence we plan to continue the program as originally proposed.

References: No peer-reviewed publications, except for abstracts (see above) resulted from the Research activities of the trainees.

Appendices: The following documents have been appended:

1. Summary of 2007 Summer Research reports

2. Abstracts of 2008 summer research projects

Summary of 2007 Research Reports

Title: A Study of *Desmodus Rotundus-Mediated* Bovine Rabies in Ometepec, Mexico
Trainee: Victoria Fields
Mentors: Dr. Louise Maranda and Dr. Joann Lindenmayer

Summary : A GPS map was constructed to describe the situation of *D. rotundus-medialed* bovine rabies in remote, rural areas of Ometepec, state of Guerrero, Mexico. Central nervous system specimens were taken after patients' death from bats and cows and underwent direct immunofluorescent testing (dFA). The bat specimens in addition underwent direct rapid immunohistochemical testing (DRIT), a new technique developed at the Center for Disease Control in Atlanta, Georgia. All cow and bat specimens tested negative for the rabies virus, although vampire bat transmitted rabies does exist in Ometepec. RT-PCR results are pending as a confirmatory test to the dFA and DRIT. *D. rotundus* intestine samples were used in a precipitin test to identify the meal source. Test results showed that the vampire bats are feeding from bovine species.

Title: Investigating the Risk of Food-Borne Transmission of Nipah Virus in Bangladesh
Trainee: Miranda Hillyard
Mentor: Dr. Jonathan Epstein

Summary: This study tests the hypothesis that human consumption of fruit that has been partially eaten by animals is a potential mechanism of transmission of Nipah virus or other zoonotic viruses that are excreted in the saliva of animals. Nipah virus, a recently emerging zoonotic virus, is carried by frugivorous bats of the genus Pteropus ("flying foxes") and it has been suggested that the consumption of raw date palm sap contaminated with flying fox saliva has led to spillover of this virus into humans. Pteropus giganteus is commonly found in Bangladesh, and is known to consume many of the same fruits that are eaten by people. Anecdotal evidence from Bangladesh describes the practice of preferential selection of partially eaten fruit that has been dropped by flying foxes (Epstein, unpublished). This pilot study has the following specific aims: 1) to determine how frequently partially eaten fruit is consumed by villagers, and to document if it is sold by vendors in village markets; and 2) to test for the presence of bat saliva and Nipah virus RNA on partially eaten fruits found under trees and in markets in districts that have been associated with Nipah virus outbreaks. We used anonymous surveys to determine the frequency of partially eaten fruit consumption and collection by vendors and private individuals. We will use PCR techniques to detect bat and viral RNA on fruits sampled from the marketplace, as well as fruits collected from underneath fruit trees near the study villages. The study was conducted in two districts of Bangladesh that have been associated with Nipah virus outbreaks: Kushtia and Faridpur. Results from this study demonstrate that seasonal fruit consumption practices can be used as a tool to characterize high risk practices that may lead to food-borne transmission of Nipah virus or other zoonotic viruses.

Title: The Serological Surveillance *of Rhinolophus megaphyllus for* Australian Bat Lyssavirus Trainee: Jennifer C. McRobbie
Mentor: Dr. Joann Lindenmayer

Summary: Bats are being increasingly recognized as a public health threat by serving as reservoir hosts for viruses which can cross species barriers and infect humans. The recent appearance of Australian bat lyssavirus (ABLV) in fruit bats (suborder *Megachiroptera)* in Australia has led to greater surveillance of these animals, which are associated with known human pathogens such as the new Hendra and Nipah viruses. It is reasonable to assume that other Australian bats which inhabit the same geographical areas may be carriers of ABLV, yet there has been limited surveillance of these species. Smaller, insectivorous bats of the suborder *Microchiroptera* might serve as potential carriers of ABLV. To determine the seroprevalence of antibodies to ABLV in the *Rhinolophus megaphyllus* species, we captured and sampled 60 specimens from Comet Mine in Queensland, Australia. We did not detect the presence of antibodies to ABLV in any of the specimens using an enzyme-linked immunosorbent assay (ELISA), Platelia Rabies II, developed for the in vitro detection of rabies anti-glycoprotein G antibodies. In order to broaden our understanding of the ecology of ABLV and identify new carrier species, further surveillance of Australian *Microchiroptera* is warranted.

Title: **A Survey of Seroprevalence of *Brucella melitensis* in Local Zimbabwean Caprines at Dimbangombe Waterford Ranch & Surrounding Communities.**
Trainee: **Shannabeth Minior**
Mentor: **Dr. Louise Maranda**

Summary: Brucellosis is a disease caused by the genus *Brucella* that affects animals and humans. It is known by farmers and researchers to cause contagious abortions in livestock, thus decreasing the reproductive efficiency and milk output of farm animals. This not only creates monetary loss for the farmer, but also has negative impact on livelihoods. Using the Rose Bengal Agglutination Test, this study tested 163 goats to determine a seroprevalence rate of 35.5% of *Brucellosis melitensis* in the resident goat herd at the Africa Center for Holistic Management (ACHM) in Zimbabwe. The specificity of the test was 75%, therefore seroprevalence could be as low as 14%. Three surrounding communities were also tested and their seroprevalence rates were 0%, this was due to the small sample size taken. Since prevalence rates are high, funds should be set aside for vaccination of the herd at ACHM.

Title: **Determining the Sensitivity and Specificity of the Participatory Disease Surveillance Method for Detection of Highly Pathogenic Avian Influenza in Yogyakarta Province, Indonesia**
Trainee: **M. Robyn**
Mentor: **Dr. Gretchen Kaufman**

Summary: Participatory disease surveillance (PDS) is a method for finding disease that relies upon the knowledge of farmers whose livelihoods are dependent upon the health of their livestock. The Government of Indonesia, (GOI), in partnership with the Food and Agriculture Organization (FAO) of the United Nations, has implemented a PDS system for detection of active Highly Pathogenic Avian Influenza (HPAI). The aim of this research was to assess the sensitivity and specificity of the Participatory Disease Surveillance (PDS) method for detection of HPAI currently being utilized by veterinarians in Yogyakarta Province, Indonesia. The sensitivity and specificity of the PDS method was determined at three levels: the clinical case definition used to diagnose HPAI, the combined clinical case definition and rapid Anigen® test when the rapid test is used to confirm a finding of active HPAI using the case definition, and the rapid Anigen® test regardless of clinical case definition results. These three levels were tested for sensitivities and specificities compared against real-time reverse-transcriptase polymerase chain reaction (rRT-PCR). A total of 129 interviews were performed by the PDS teams, resulting in the collection of 236 chicken samples for rRT-PCR testing. Of the 129 interviews, 34 were found to be case definition positive for HPAI (26.4%), 33 were found to be rapid test positive (25.6%), and 30 were found to be positive using the combination case definition + rapid test method (23.3%). Using the H5 method rRT-PCR results, the clinical case definition alone was found to have a sensitivity of 44.2% and a specificity of 92.3%. The Anigen® test alone was found to have a sensitivity of 42.9% and a specificity of 98.1%. The combination of the clinical case definition and Anigen® test was found to have a sensitivity of 39.0% and a specificity of 98.1%. These results show that there is a high level of congruity between the clinical case definition and the rapid Anigen® test. It appears that the current utilization of the PDS method in Yogyakarta Province may be missing a number of cases of HPAI, however, the cases which are diagnosed as HPAI are highly likely to be the disease and veterinarians should not shy away from taking appropriate control measures based on a positive PDS diagnosis.

Title: **Comparing the Diagnosis of Bovine Tuberculosis in Nasal Exudates and Milk Samples Using Polymerase Chain Reactions and the Simple Caudal Fold Tuberculin Test**
Trainee: **Rebecca Steers**
Mentors: **Dr. Camila Arriaga Díaz, Dr. Cecilia Romero Torres, and Dr. Feliciano Milian Suazo**

Summary: Despite the current eradication efforts, bovine tuberculosis (TB) continues to be a problem in Mexico as it persists in the dairy cattle industry. The purpose of this study was to investigate the use of nested

polymerase chain reaction (PCR) as a *pre-mortem* diagnostic tool, utilizing nasal exudates or milk as clinical samples in an area with a high prevalence of TB. This was done by analyzing the agreement between the caudal fold tuberculin (SCFT) test and the nested PCR results. The PCR analysis focused on the gene for the MPB70 protein. Using nasal exudates as a clinical sample, the nested PCR detected 18 positive cases and 18 negative cases. Although this was the same number of positive and negative cases as the SCFT test, the two tests did not detect all of the same cases and therefore had poor agreement (k = 0.112). Using milk as a clinical sample, the nested PCR detected 5 positive cases and 31 negative cases. The calculated Kappa value showed poor agreement with the SCFT test (k = 0.166). Suggestions are made in this report on how to improve upon the study in order to confirm the agreement between the two tests.

Title: **Development of a PCR diagnostic technique to differentiate *Mycobacterium tuberculosis, Mycobacterium bovis* and *Mycobacterium avium* in captive elephants in Nepal**

Trainee: **Tierra Wilson**
Mentors: **Dr. Gretchen Kaufman, Dr. Jean Mukherjee and Dr. Donna Akiyoshi**

Summary : Tuberculosis poses a significant threat to captive elephants in the United States and in developing countries. In Nepal, elephants are in close physical association with cattle and humans providing opportunity for bidirectional transmission *of Mycobacterium tuberculosis* and *Mycobacterium bovis* between elephants, cattle and humans. Current diagnosis of tuberculosis in elephants relies on trunk wash culture, which takes 8-12 weeks, is labor intensive and does not have good sensitivity. Serological diagnostic techniques are in use but are used in conjunction with culture. In many cases, these tests do not effectively differentiate between the three types *of Mycobacterium* likely present, *Mycobacterium tuberculosis, Mycobacterium bovis* and *Mycobacterium avium.* This project developed a PCR based method for differentiating *Mycobacterium tuberculosis, Mycobacterium bovis,* and *Mycobacterium avium* using the gyrB gene. This method proved to be effective in differentiating *Mycobacterium* species in laboratory isolates but was unable to detect *Mycobacterium* DNA in trunk washes from Nepali elephants suspected to be *Mycobacterium-positive.* Taq polymerase inhibitors were found in the dirt/debris present inside elephant trunks and a method for the removal of dirt/debris was devised. Further investigation is needed to improve the removal of inhibitors and to increase the recovery *of Mycobacterium* DNA from trunk washes. The development of a more accurate and efficient test for tuberculosis is urgently needed to improve detection and opportunities for successful treatment, decrease the likelihood for development of multi-drug resistant strains and to reduce the transmission of infection to people and other elephants. The multiplex PCR developed in this study has the potential to be this needed diagnostic test for tuberculosis because it has the sensitivity and ability to differentiate the three *Mycobacterium* species most commonly associated with tuberculosis infections in elephants.

Summary of 2008 Proposed Summer Research

Awardee: Karen Alroy V'11
Mentor: Dr. Julie Ellis
Research Project: Herring Gulls, *Larus argentatus*, as vectors of pathogenic *Escherichia coli* in Coastal Environments.

Summary: This study will investigate the potential for Herring Gulls (*Larus argentatus*) to transmit anthropogenically-derived, pathogenic bacteria to marine vertebrates and humans in coastal habitats at Cape Cod, MA. Strains of *Escherichia coli* from gulls, seals, wastewater, and landfill trash will be compared through genotypic microbial source tracking (ribotyping); isolates will also be serotyped in order to assess potential pathogenicity. I will record behavioral interactions between gulls and seals and between gulls and humans in order to characterize the possible routes of bacterial transmission between species. Cape Cod is a major vacation destination on the Atlantic coast, and beach closures due to elevated fecal coliform counts are common during the summer months. Results from this study will contribute significantly to a greater understanding of the sources of fecal pollution at Cape Cod beaches.

Awardee: Sarah Carter V'10
Mentor: Dr. Gretchen Kaufman
Research Project: Determining Geographic Risk Factors for Disease Transmission between Livestock and Elephants in Chitwan, Nepal.

Summary: Royal Chitwan National Park in the Terai region of Nepal is surrounded by a buffer zone whose resources are used by livestock, humans, captive elephants and wild animals. The shared use of this landscape creates the opportunity for disease transmission between these populations. Previous research has shown that Fasciola spp is present in both the livestock and captive elephant populations. It is also established that tuberculosis infects captive elephants, cattle and humans in the area. A previous study mapped the area used by captive elephants in the buffer zone around the park. The proposed study will investigate the characteristics of these elephant ranges to determine the regions within them that present the highest risk for disease transmission between elephants and livestock. GPS and transect methodologies will be used to record data on dung counts and animal observations. It will also map areas that have the required habitat for Lymnaea snail development, the host of the Fasciola fluke. Dung counts, animal observations and Lymnaea snail habitat will be mapped using GIS. By comparing landscapes used by at risk species with areas that are favorable habitat for the Fasciola fluke, an idea of where disease is most likely being spread can be developed. This information can then be used to inform management decisions to decrease the spread of diseases shared between elephants and livestock.

Awardee: Philip Gerwin V'11
Mentor: Dr. Phyllis Mann
Research Project: The Role of Progesterone and Its Metabolites on the Onset of Maternal Behavior during Pregnancy in Rats.

Summary: Understanding maternal behavior has important applications to both social and public health. While much is known about the hormones of pregnancy, little is known about where they act, their mechanisms of action and how they inhibit or stimulate the display of maternal behavior. This research project aims to elucidate these mechanisms of inhibition and stimulation in both the ventromedial nucleus of the hypothalamus (VMM) and medial preoptic area (MPOA), respectively. The VMM is known to be part of an inhibitory neural circuit that regulates maternal behavior. It is believed that progesterone may be involved in this inhibitory mechanism and that's its withdrawal just prior to parturition leads to the disinhibition of maternal behavior. It has also been suggested that some of progesterone's actions may be due to its reduced metabolites, including allopregnanolone. This experiment will explore the role of progesterone metabolites and will look at the MPOA as well as the VMM. Cannulas attached to osmotic minipumps will be placed into the VMM in one set of rats and the MPOA in another on day 11 of pregnancy containing either finasteride,

allopregnanolone, or vehicle. Maternal behavior testing will begin on day 15 and continue until the primigravid rat displays maternal behavior. The rats will also be tested in the elevated plus maze (EPM) on day 15, which is the gold standard for testing for anxiety by measuring time spent in the open arms of the maze. Parturition usually occurs on day 22-23. Body weight and litter data will be collected one day after parturition. The brain will also be collected to validate placement of the cannulas.

Awardee: Sara Heslop V'11
Mentor: Dr. Louise Maranda
Research Project: Prevalence of Gastro-intestinal Parasites in White-faced Capuchin Monkeys, *Cebus capuchinus,* in Heavily Touristed National Parks in Costa Rica Compared to Isolated National Parks.

Summary: I would investigate whether white-faced capuchin monkeys, *Cebus capuchinus*, in Manuel Antonio National Park, Costa Rica experience a higher prevalence of gastro-intestinal parasitic infection than white-faced capuchin monkeys in the more isolated park of Corcovado National Park. This will be examined by collecting fecal samples from monkeys at both parks, determining the prevalence and comparing the levels using the chi square test for significance. This study will determine whether there is an association between the prevalence of gastro-intestinal parasites observed in the monkeys and the level of tourism experienced by the park. This will help to establish a baseline for prevalence of parasitic infection which could be expanded upon in future studies.

Awardee: Miranda Hillyard V'10
Mentor: Dr. Robyn Alders
Research Project: Aflatoxicosis in Indonesian Domestic Ducks: Implications for H5N1 Highly Athogenic Avian Influenza (HPAI) Vaccination Campaigns.

Summary: Highly pathogenic avian influenza A (HPAI) subtype H5N1 is now endemic in Indonesia. HPAI poses a serious threat to both poultry industry and to human health in Indonesia. Indonesia is considered the most likely source of a human influenza A pandemic. Vaccination in poultry is widely used to slow the spread of the disease and prevent human infection. Vaccination programs have focused primarily on chickens in the past but are currently expanding to include domestic ducks. Ducks serve as an important reservoir for the virus and contribute to its spread. Successful vaccination depends on a competent immune system. Aflatoxin is a mycotoxin produced by *Aspergillus* sp. molds and is commonly found in commercial livestock feeds. Ducks in Indonesia could be immunosuppressed due to aflatoxin exposure. This study will test for aflatoxicosis in ducks and determine the implications this has for H5N1 vaccination and control programs. *Specific Aim 1:* To determine the prevalence of aflatoxicosis in domestic ducks in central Java, Indonesia. *Specific Aim 2:* To evaluate whether aflatoxicosis could result in vaccine failure in domestic ducks.

Awarded: Lauren O'Connell V'11
Mentor: Dr. Robert Bridges
Research Project: Estrogen, Maternal Behavior and Stress

Summary: Using the Elevated Plus Maze, it has been demonstrated that rats that are young cycling first time mothers show lower levels of anxiety like behavior during proestrus when compared to age-matched females who had not yet had a litter (Byrnes and Bridges 2006). This test has also been used to illustrate that rats who have been treated with exogenous estradiol and prolactin also exhibit lower levels of anxiety like behavior when compared to untreated age matched controls. (Frye and Waif, 2004; Lund et al. 2005; Marcondes et al. 2001; Torner et al. 2001,2002). While estradiol levels are low in pregnancy, the majority of studies examining the effects of estradiol on anxiety demonstrate that increasing levels of estradiol are associated with decreased anxiety-like behavior. One possible explainaion for this discrepancy is that parity alters the relative levels of estrogen receptors(ER), ERa and ER |3. This proposed experiment would set out to determine the role of ERa in the reduction of anxiety-like behavior observed in reproductively experienced females. One week after being

ovariectomized, all 32 rats and their aged matched nulliparous females will be treated with either the ERa agonist PPT (0.01, 0.1, or 1 mg/kg, subcutaneously) or vehicle (DMSO). Thirty-minutes post injection these animals will be tested on the Elevated Plus Maze to determine their level of anxiety-like behavior. A body temperature will be taken, and the rats will be sacrificed. Blood, anterior pituitary, and brain will be collected for later testing.

Awardee: Kathleen Riley V'10
Mentor: Dr. Gretchen Kaufman
Research Project: Diagnosis and Treatment of Mange in the Street Dog Population of Kathmandu, Nepal.

Summary: For the upcoming summer, I plan to work with the Kathmandu Animal Treatment Centre in Kathmandu, Nepal, to address the problem of cutaneous mite infestation, commonly called mange, in the stray dog population served by their Animal Birth Control/Anti-Rabies program. Mange is an extremely common problem in the stray dogs brought to KAT, and can compromise their ability to heal from sterilization surgery, as well as creating permissive conditions for the transmission of infectious bacterial diseases and contributes significantly to the overall suffering and ill health of these animals. My research plan is to compare two techniques for collecting samples for definitive diagnosis of mange by light microscopy: to determine whether the less invasive hair plucking technique is comparable to deep skin scraping for positive diagnosis. I will also use this data to compare the prevalence of mange-causing mite species, *Demodex* spp. and *Sarcoptes scabiei*, and statistically evaluate the relationship of factors such as age, sex, weight, body condition, and capture location with mite species and severity of clinical signs. Determining the type of mange is extremely important, as *Demodex* is not generally contagious, whereas *S. scabiei* is spread by contact, and dog handling and housing can be important control points to prevent transmission. In dogs with a positive diagnosis of sarcoptic or demodectic mange, treatment will be undertaken, either with current methods of full body amitraz or lime-sulfur dips, or with newer spotted-on metaflumizone/amitraz treatment (ProMeris, Fort Dodge Animal Health). The efficacy of each treatment will be evaluated daily based on visible signs, and microscopically at the end of treatment at KAT, usually one week. Treatment data will be analyzed based on mite species, initial severity, and other factors noted at admission. The end goal of this study is to quantify the prevalence of demodectic and sarcoptic mange in the stray dogs served by KAT, determine whether mite species or other factors predict differential responses to treatment, and compare spotted-on treatments to established whole-body treatments in this field setting.

Awardee: Annie Shea V'11
Mentor: Dr. Scott Shaw
Research Project: The Use of Directed Education to Improve the Rate of Hand Washing in a Veterinary Hospital.

Summary: Since Semmelweis first made the observation in 1846 that disease transmission could be reduced with good hand washing technique between patients, human and veterinary hospitals have struggled to improve health care provider compliance with frequent and consistent hand washing and, more recently, the use of antibacterial hand rubs.

Previous work by Dr. Scott Shaw has shown that 11% of dogs in the Intensive Care Unit of the Foster Hospital for Small Animals develop a hospital-acquired infection and 1/100 dogs admitted to the ICU die as a result of hospital-acquired infections. There is extensive data in human hospitals that increasing hand washing compliance can reduce the rates of hospital-acquired infections. We hypothesize that a multi-modal, inclusive, long-term educational campaign can significantly increase hand washing behavior among hospital employees at Tufts.

We plan to evaluate the rates of hand washing and/or use of antibacterial hand rubs among technicians, students and veterinarians in the Intensive Care Unit and in the wards of the Foster Hospital for Small Animals, plan and implement an educational campaign to increase the rates of hand washing and/or use of antibacterial hand rubs, and finally, re-measure compliance post implementation of the campaign. We plan to involve staff members at different positions and levels in planning and implementing an educational campaign. The plan

will include long-term, periodic re-evaluations and if necessary, repeat workshops. Although there is extensive data on rates of hand washing compliance and rates of improvement using various techniques in human hospitals, there is scant similar data for animal hospitals. This project will provide baseline data on hand washing compliance and provide a model educational program for improving compliance that could potentially be integrated at other veterinary hospitals.

Awardee: Angela Snell V'11
Mentor: Dr. Robyn Alders
Research Project: A Participatory Approach to Understand Poultry Farmer Perceptions of Avian Influenza (H5N1).

Summary: Vaccination against diseases of economic importance is commonplace in the Indonesian commercial poultry industry. The use of vaccination in backyard poultry has been and continues to be problematic. There has been little engagement by the Ministry of Agriculture with poultry producers and this is reflected in the absence of a coherent disease control strategy. The H5N1 virus subtype of Highly Pathogenic Avian Influenza (HPAI) in Indonesia undergoes frequent mutation and the lack of a comprehensive vaccination strategy that includes monitoring of field strains has at times left poultry vulnerable to infection. The Operational Research in Indonesia for More Effective Control of Avian Influenza (ORI HPAI) program will employ the vaccine strain that has demonstrated the best protection against local isolates of the H5N1 strain in Indonesia. Participatory Disease Surveillance and Response (PDS/R) teams working with farmers raising backyard poultry will use the vaccine in a longitudinal study in an effort to improve control of the virus. The FAO has stated that a long-term approach is necessary in order to reduce the national viral load. Such an approach requires a high degree of participation and compliance from poultry farmers throughout the country. The implementation of ineffective vaccination programs in the past is believed to have impacted farmer perception of national control programs. Additionally, it is known that national control programs have had little interaction with commercial producers, whose flocks contribute significantly to high viral load in certain areas of the country. For livestock disease control in general, farmer perceptions of disease risk and the benefits and risks of disease control options are important criteria in farmers' decisions. Because farmer perceptions play an important role in the ultimate success of disease control programs, this study will focus on farmer perceptions in four key areas: origin of HPAI, threats associated with HPAI, vaccine efficacy, and vaccination protocol. Questionnaire surveys will be carried out in areas where the longitudinal study is being conducted using a Participatory Learning Appraisal (PLA) technique. This technique will permit systematic coverage of issues for cross-site comparisons. Qualitative/semi-quantitative insights on farmer perceptions will be obtained, analyzed, and shared with the Indonesian Ministry of Agriculture and other stakeholders in the ORI HPAI program.

Awardee: Christine Taylor V'11
Mentor: Dr. Peter Daszak
Research Project: Investigating the Emergence of SARS and SARS-like Coronoviruses.

Summary: Emerging infectious diseases (EIDs) are diseases that move from one population to another or rapidly increase in incidence. EIDs are the leading cause of death worldwide, accounting for the deaths of 13 million people per year. Despite the social, demographic, and economic impact of EIDs and the billions of dollars spent on drug and vaccine development to control them, the process of disease emergence remains poorly understood (Daszak et al. 2000; Thanassi & Schoen 2000; Hotez et al. 2004). In the case of SARS our knowledge of the ecology of the reservoir host is limited and key data on the interactions among the host species, incident carriers, and humans is necessary to be able to predict and prevent future disease emergence. The focus of this proposal is to examine the ecology of SARS and SARS-like coronviruses (SL-CoV) in insectivorous bats of the genus Rhinopholus, and using comparative ELISA screening, to investigate cross species transmission and emergence of SL-CoV in wild and wild caught animals from live animal markets the Guangdong province of southern China.

Awardee: Stephen Wilson V'11

Mentor: Dr. Jon Epstein
Research Project: Infectious Risks of Brushmeat Hunting on Malaysia

Summary: This study is part of a collaboration between the Consortium for Conservation Medicine (CCM) and the National Public Health Laboratory, under the Malaysian Ministry of Health, to assess the risk of zoonotic pathogen transmission from wildlife to hunters and restaurant workers who are shooting and butchering wildlife as part of the bushmeat trade in Peninsular Malaysia. This will give insight into the frequency and variety of species these hunters contact, and by further examination of their methods of hunting and butchering the meat, characterize the possible routes of exposure to wildlife pathogens via bodily fluids. In addition to hunting, we will examine the transport of animals to restaurants and the preparation of bushmeat before being served. By studying hunting and butchering behaviors, an assessment can be made of the highest risk transmission points between wildlife and humans. Based on this assessment, we aim to develop a practical and easily adoptable education program designed to improve the safety of those handling and butchering wild animals. This may reduce the risk of transmission of zoonotic pathogens thereby reducing the risk of an outbreak, by protecting those who are most at risk of initial contact with a potential pathogen.

In addition to the survey, I will work closely with the National Public Health Laboratory, to develop protocols for testing dried blood spots for pathogens, beginning with Nipah virus. The Nipah virus has been selected for initial testing, as it is harbored in pteropid bats, an animal heavily hunted in Malaysia as it is considered a delicacy among Malaysia's large Chinese and Manadonese populations (Li et al., 2005; Fujita, 1998). This testing will be done using an already amassed collection of blood spots on filter paper from bushmeat collected by hunters as part of an existing collaborative project run by the CCM (Epstein, PI) looking at the rate of exposure to various zoonotic pathogens through bushmeat hunting. RNA extraction protocols as well as a PCR protocols will be tested to determine whether it is possible to detect the presence of pteropid bat RNA and Nipah virus RNA in dried blood from filter paper, which has been done in other studies (Fiscus et al., 1998). This test will be used to determine the prevalence of Nipah virus in the bushmeat collected and thus the rate of exposure to the virus can be assessed. This RNA extraction can eventually be used to test both hunters' and food handler's blood for Nipah virus, as well as other suspected RNA viruses.

Awardee: Tierra Wilson V'10
Mentor: Dr. Jean Mukherjee
Research Project: Development of a Saliva Collection Technique for Improved Health-Monitoring of Free-Living Mountain Gorillas.

Summary: The mountain gorilla (*Gorilla gorilla beringei*) is listed as endangered by The World Conservation Union and exists in only small isolated areas of Rwanda, Uganda and Democratic Republic of Congo. The population consists of around 700 gorillas, which are currently being threatened by habitat encroachment, poaching and exposure to human diseases. Measles, tuberculosis, and ebola are among the most serious and exist in the human populations surrounding the gorilla's habitat. The mountain gorilla veterinary project (MGVP) provides emergency medicine and pathology services to the mountain gorilla population. Health monitoring is done with minimal intervention through direct observation and collection of fecal samples for diagnostic screening (4). This non-invasive method is most beneficial to minimize stress however it makes early detection and diagnosis of disease outbreaks difficult because many pathogens are not detectable in feces. There are currently two recurring disease outbreaks within the population that are not being diagnosed and need further investigation. One is of respiratory origin and the other consists of skin lesions on the nose and mouth.

This project aims to develop a non-invasive technique to collect saliva from free-living mountain gorillas for diagnostic testing. Saliva diagnostics have the potential to diagnose endemic diseases as well as provide early surveillance for more threatening diseases that could affect the population in the future. All fieldwork will be completed in the Parc National des Volcans and the MGVP orphan gorilla facility in Kinigi, Rwanda. Subjects will include the orphans at the MGVP center and free-living habituated gorillas in the park. Three collection methods will be evaluated; natural collection from discarded food items, a dental rope method and a mesh box method. Validation of saliva collection will be done using a Phedebas Forensic Press Test

(Phedebas Forensics) and QuantiChrom alpha-amylase assay kit (Bioassay Systems). The three saliva collection techniques will be evaluated based on a 0 to +3 visual scoring of alpha-amylase tests, ease of sample collection, and consistency of saliva volume. Samples will also be evaluated for the presence of cortisol using an enzyme immunoassay (Salimetrics LLC) to determine if hormones can be recovered from these samples. Samples will be stored on FTA cards and shipped to the U.S.A for future PCR testing.